REMARKS:

Applicants express gratitude to the Examiner for useful discussions during an interview on May 6, 2010. The preceding amendments and following remarks form a full and complete response to the office action dated January 13, 2010. Claims 8, 9, 16, 18, 27, 30-33 have been amended and new claims 34-37 have been added. New claim 34 recites subject matter deleted from claims 8 and 9. New claims 35 and 37 recite subject matter deleted from claim 27. No new matter has been added.

Interview Summary

During an interview on May 6, 2010, Applicant's representative presented arguments and cited sections in WO 09/040134 to show that antibodies to HB-EGF inhibit proliferation both in vitro and in vivo. The Examiner stated that she was willing to reconsider the enablement of antibodies in vivo based on this evidence. Applicant's representative also presented arguments with respect to other types of inhibitors and showed correlation between in vitro and in vivo using siRNA and EGFR. The Examiner was concerned with the claims being directed to inhibition of the EGFR ligand vs. the reference showing agents acting on the EGF receptor. Applicant's representative was asked to address this concern in this response and to reiterate arguments with respect to other ligand inhibitors.

Response to Rejections under 35 U.S.C. § 112

Claims 2, 4-9, 15-27, 30-33 were rejected under 35 U.S.C. § 112, first paragraph as lacking enablement. The Examiner maintained that while the specification is enabling for a method of treating hyperproliferative cells *in vitro* comprising the

administration of an inhibitor of a receptor tyrosine kinase ligand, it does not provide enablement for a method of treating hyperproliferative cells *in vivo* comprising the administration of an inhibitor of a receptor tyrosine kinase ligand. The Examiner also asserted that the issues raised in the rejection regarding enablement of "prevention" and "gene therapy" were not addressed.

As discussed during the interview on May 6, 2010, Applicants provided support for the correlation between in vitro results in the specification and the in vitro and in vivo data presented in the applicant's work in WO 09/040134. The Applicants submit herewith a declaration by co-inventor, Dr. Zwick-Wallasch, detailing the correlation. In her declaration, Dr. Zwick-Wallasch states that the present application application discusses a number of receptor tyrosine kinase ligand inhibitors including antibodies or antibody fragments, proteinaceous or low-molecular weight inhibitors, and siRNAs (on pages 8 and 9 of the specification).

The examples in this application are not limited to siRNAs, but rather, the effective inhibition of tyrosine kinase ligands is additionally demonstrated using inhibitors of HB-EGF, such as CRM197, which is a protein, and compounds which inhibit the release of EGF and EGF-like ligands such as batimastat (BB94), which is a low molecular weight metalloproteinase inhibitor. In accordance with Examiner Huff's suggestion to further clarify the claims, independent claims 30-33 have been amended by inserting the recitation "wherein said inhibitor acts directly on said receptor tyrosine kinase ligand itself or on a metalloprotease capable of cleaving said receptor tyrosine kinase ligand." Support for this amendment can be found on page 6, lines 28-30 of the specification. Furthermore, claims 30 and 33 have been amended to remove the word

"preventing." Thus, Applicants submit that the enablement issues with regard to "prevention" have been rendered moot.

By inhibiting the release of EGF and EGF-like ligands, applicants demonstrate the inhibition of EGFR activation. The experimental data illustrated by Figures 3 and 4 and example result sections 2.3 and 2.4 relate to analysis of proHB-EGF release in response to stress agents and blockage of HB-EGF release by inhibitors. The data shows that stress agents induce phosphorylation of EGFR, and that inhibitors which inihibit HB-EGF or the release of tyrosine kinase ligands (EGF-like ligands) effectively block this phosphorylation (section 2.3 on pages 22-23 of the specification). The immunoblots show that inhibitors of HB-EGF (CRM197) and those which inhibit the release of EGF-like ligands (BB94) inhibit EGFR activation (Figure 3) and that is was shown that these inhibitors block HB-EGF release (Figure 4). An anti-HB-EGF antibody is used in the HB-EGF release assay demonstrating its binding to proHB-EGF (Figure 4a).

Dr. Zwick-Wallasch's declaration provides evidence of the correlation of the in vitro results demonstrated in the present application with in vivo efficacy as demonstrated in WO 09/040134. Dr. Zwick-Wallasch states that the *in vitro* data in the present application shows that EGFR activation and release of HB-EGF is blocked by a specific inhibitor of HB-EGF (CRM197) and by a metalloprotease inhibitor (BB94), which inhibits HB-EGF and the release of HB-EGF and other EGF-like ligands, in several human tumor cell lines and that anti-HB-EGF antibodies do indeed bind to proHB-EGF (see the blot showing that anti-HB-EGF bound to HB-EGF in Figure 4b).

WO 09/040134 states that "[w]hether an amino acid change results in a functional antibody, i.e., in a antibody that binds to HB-EGF and reduces, neutralizes or substantially inhibits the function of HB-EGF, can readily be determined by assaying the specific activity of the resulting antibody in ELISA or FACS for binding to HB- EGF or *in vitro* or *in vivo* functional assay," (paragraph [000207]). Further, the *in vitro* data in WO 2009 that shows that Anti-HB-EGF antibodies inhibit EGFR phosphorylation in human tumor cells (see paragraphs [000302-000305] and Figure 22, 23 and 24), as well as *in vivo* data showing that anti-HB-EGF antibodies cause reduced pancreatic tumor (Fig. 37) and ovarian cancer tumor growth (Fig. 38A-C) in mice. It is Dr. Zwick-Wallasch's expert opinion that the data in WO 2009 demonstrates that the HB-EGF inhibitor CRM197 as well as anti-HB-EGF antibodies block EGFR activation in Cos-7 cells stimulated by the G-protein-coupled receptor ligand LPA (Figure 23), which leads to extracellular processing of transmembrane growth factor precursor and release of mature growth factor, which interacts with the ectodomain of the EGFR and activates it through tyrosine phosphorylation (Prenzel et al., 1999, Nature 402:884-888). Thus, because this application shows binding of anti-HB-EGF antibodies to HB-EGF (Figure 4a) and that inhibitors of HB-EGF and those which inhibit the release of HB-EGF and EGF-like ligands block the release of HB-EGF in Cos-7 cells (Figure 4) and inhibit activation and phosphorylation of EGFR in Cos-7, TCC-Sup and NCI-H292 cells,(Figure 3) and WO 2009 shows that an inhibitors of HB-EGF, namely anti-HB-EGF antibodies bind to HB-EGF and inhibit activation and phosphorylation of EGFR in LPA-stimulated Cos-7 cells (Figure 23) and also shows that this same inhibitor reduces tumor growth in

mice, there is clearly a correlation between the *in vitro* results presented in this application and the *in vitro* and *in vivo* results shown in WO 2009.

Therefore, as discussed during the May 6th interview, it appears that a correlation between the *in vitro* experiments in the cos-7 cells, cancerous cell lines provided in this application (Figures 3 and 4) and the cos-7 cells, the cancer cell lines, and the mouse *in vivo* models shown in WO 09/040134 (see, e.g., paragraphs [000303-000319] for *in vitro* and paragraphs [000375] for *in vivo*), should be recognized.

In addition to the above, Applicants submit that claims 26, 27, 35, and 36 are directed to the method limited to inhibitors that act on the protein level such as a specific protein inhibitor, an antibody or antibody fragment directed against a tyrosine kinase ligand, e.g., HB-EGF. Based on the showing of the correlation of *in vitro* and *in vivo* inhibition using antibodies, Applicants submit that the present claims are enabling for the claimed method of administering inhibitors that act on the protein level and respectfully request an indication of allowance for these embodiments.

Further, the examiner requested that applicants reiterate their arguments for other ligand inhibitors. Applicants submit that claim 37 is directed to the method limited to administering a proteinaceous or low-molecular weight inhibitor. In the present application, the experimental data illustrated by Figures 3 and 4 and example result sections 2.3 and 2.4 relate to analysis of proHB-EGF release in response to stress agents and blockage of HB-EGF release by inhibitors. The immunoblots show that inhibitors of HB-EGF (CRM197) and those which inhibit the release of EGF-like ligands (BB94) inhibit EGFR activation (Figure 3) and that is was shown that these inhibitors

block HB-EGF release (Figure 4). The enclosed reference by Ichise et al. shows the in vitro and in vivo efficacy of administering Crm197 (a proteinaceous EGF inhibitor) on cancer cells (see Figure 2C) and in tumor growth in a humanized knock-in xenograft mouse model (Figures 6 A-C (hz/hz) and Figure 7). The enclosed reference by Durkin et al. shows the correlation of in vitro and in vivo experiments with batimastat (BB94) – referred to as matrix metalloproteinase inhibitor (MMPI) in Durkin et al., shows that batimastat decreased tumor weights and volumes compared to a control (see Figure 6) and significantly increased survival rates in a murine model of pancreatic adenocarcinoma (see Figure 4). Thus, based on the showing of a correlation of in vitro and in vivo efficacy using these tyrosine kinase ligand inhibitors, Applicants submit that the present claims are enabling for the claimed method of administering proteinaceous and low molecular weight tyrosine kinase ligand inhibitors and respectfully request an indication of allowance for these embodiments.

With regard to the Examiner's request for a showing of correlation between the in vitro siRNA data and in vivo efficacy, Applicants submit herewith a paper by Zhang et al. published in 2004, entitled "Intravenous RNA Interference Gene Therapy Targeting the Human Epidermal Growth Factor Receptor Prolongs Survival in Intracranial Brain Cancer," which shows the state of the art recognizing a correlation between in vitro efficacy and in vivo effectiveness of using siRNAs for gene therapy.

In her declaration, Dr. Zwick-Wallasch states that the Zhang et al. paper provides an excellent demonstration of the correlation of *in vitro* assays with *in vivo*

efficacy with regard to the use of EGFR inhibitors, specifically siRNAs, for gene therapy treatment of cancer.

Zhang et al. detail *in vitro* and *in vivo* experiments, wherein, in cultured glioma cells, the delivery of the RNAi expression plasmid resulted in a 95% suppression of EGFR function, and weekly i.v. RNAi gene therapy caused reduced tumor expression of immunoreactive EGFR and an 88% increase in survival time of mice with advanced intracranial brain cancer (abstract). The first paragraph of the discussion section on page 3673 states that "[t]he results of these studies are consistent with the following conclusions. First, it is possible to knock down *EGFR* gene expression with i.v. gene therapy that uses expression plasmids encoding a shRNA directed at nucleotides 2529–2557 of the human EGFR mRNA (Table 2)." This shows the *in vitro* effects of an inhibitor of a receptor tyrosine kinase ligand in cells. Further, this data demonstrates successful gene therapy ***in vitro***. Further, "EGFR expression knockdown is demonstrated by the inhibition of thymidine incorporation or calcium flux in human U87 glioma cells in tissue culture (Tables 2 and 3; Fig. 3), by the decrease in the expression of immunoreactive EGFR in cell culture (Fig. 2)." Thus, the *in vitro* effect of an inhibitor of a receptor tyrosine kinase ligand is demonstrated **in tissue**. Correlation of the *in vitro* results with *in vivo* effectiveness is also provided in the same paragraph based on the disclosure that there is a "decrease in brain cancer expression of immunoreactive EGFR *in vivo* (Fig. 6). Third, anti-EGFR gene therapy has an antiangiogenic effect and results in a 72–80% decrease in vascular density of the tumor (Fig. 5; Table 4)." With regard to gene therapy ***in vivo***, the same paragraph states that "weekly i.v. anti-EGFR

gene therapy results in an 88% increase in survival time in adult mice with intracranial brain cancer (Fig. 4)."

According to MPEP § 2164.02,

> if the art is such that a particular model is recognized as correlating to a specific condition, then it should be accepted as correlating unless the examiner has evidence that the model does not correlate. Even with such evidence, the examiner must weigh the evidence for and against correlation and decide whether one skilled in the art would accept the model as reasonably correlating to the condition. *In re Brana*, 51 F.3d 1560, 1566, 34 USPQ2d 1436, 1441 (Fed. Cir. 1995) (reversing the PTO decision based on finding that *in vitro* data did not support *in vivo* applications).

Zhang et al. was published prior to the U.S. filing of this application so its teachings should be considered as having been recognized by those of ordinary skill in the art. The Examiner was concerned that the Zhang et al. results related to the effective use of siRNAs and gene therapy targeting the EGF receptor rather than EGF ligand as is the subject of the present invention. Applicants submit that if the art recognizes a correlation between in vitro and in vivo knockdown of the EGF receptor using siRNAs, it follows that knockdown of the EGF receptor activator, i.e., EGF ligand, can also be reasonably expected to have an *in vivo* effect. Applicants are not aware of evidence showing that gene therapy using siRNAs against a receptor tyrosine kinase ligand itself or on a metalloprotease capable of cleaving said receptor tyrosine kinase ligand *in vitro* does not correlate with *in vivo* results. Thus, Applicants respectfully submit that the evidence for a correlation based on Zhang et al. should be given considerable weight in the absence of contrary evidence.

Zhang et al. has shown that knocking down the EGF receptor, i.e., by causing a "95% suppression of EGFR function (abstract), increases survival time and reduces

tumor vascular density (see page 3673, first paragraph of Discussion). Because the present application shows that "EGFR activation in response to osmotic and oxidative stress is ligand-dependent" (page 14, lines 5-6 and Figure 3), and Applicants have used siRNAs to specifically block expression of the ADAM family metalloproteases, which cleave receptor tyrosine kinase ligands, allowing them to bind to and activate EGFR (see Figure 5), Applicants believe that a reasonable correlation can be expected between the *in vitro* results shown here and *in vivo* experiments when siRNAs are used to silence the ligands that activate EGFR. Thus, based on the above, Applicants submit that the state of the art in 2004 recognized a correlation between in vitro and in vivo efficacy in cancer cell lines and mouse models. Applicants therefore submit that the in vitro results shown herein do reasonably enable one of ordinary skill to use the presently claimed method and respectfully request that the rejections under 35 U.S.C. § 112, first paragraph, be withdrawn.

Based on the above, Applicants submit that the present specification enables one of ordinary skill to use the presently claimed method, not only by administering antibodies or antibody fragments directed against a tyrosine kinase ligand, but also proteinaceous inhibitors, low molecular weight inhibitors, and also siRNAs. Thus, Applicants respectfully request that the rejections under 35 U.S.C. § 112, first paragraph, be withdrawn.

Conclusion

In view of the above amendments and remarks hereto, Applicants believe that all of the Examiner's rejections set forth in the January 13, 2010 Office Action have been fully overcome and that the present claims fully satisfy the patent statutes. Applicants,

therefore, believe that the application is in condition for allowance. The Director is authorized to charge any fees or overpayment to Deposit Account No. 02-2135.

The Examiner is invited to telephone the undersigned if it is deemed to expedite allowance of the application.

Respectfully submitted,

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Enclosures: Declaration of Dr. Zwick-Swallasch, Zhang et al., Durkin et al., Ichise et al.
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